DESCRIPTION

METHOD OF JUDGING BIOLOGICAL ACTIVITY IN BIOREMEDIATION SITE AND POLYNUCLEOTIDE FOR DETECTING MICROORGANISM TO BE USED THEREIN

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Technical Field

[0001] The present invention relates to a method of judging a biological activity at a bioremediation site and a polynucleotide for detecting a microorganism used therein.

Background Art

[0002] Contamination of groundwater and soil with various types of organochlorine compounds including tetrachloroethylene (PCE) and trichloroethylene (TCE) has been a serious issue around the globe. This issue often has been covered thoroughly by mass media such as newspapers and the like, leading to a strong social demand for development of technologies for remedying environments contaminated with these substances.

[0003] Such technologies for remedying a contaminated environment include a physicochemical method and a biological method. Among these methods, particularly suited for remediation with respect to low-level contamination is the biological method of remedying an environment using a microorganism (bioremediation). Bioremediation is widely expected to be put to practical use since it can be performed at low cost without requiring soil excavation, allows even an environment under a building to be remedied easily, and

achieves a reduction in environmental burdens.

[0004] Bioremediation is performed in the following manners. That is, for example, various nutritive substances or the like are supplied to microorganisms originally inhabiting contaminated soil or groundwater so that the capability of the microorganisms to degrade and eliminate environmental contaminants is enhanced (biostimulation). Alternatively, microorganisms having the capability to degrade and eliminate environmental contaminants are introduced directly into a contaminated environment (bioaugmentation: JP 2003-154332 A, for example). An example also is known in which biostimulation and bioaugmentation were used to remedy an environment that was groundwater contaminated with TCE, yielding excellent results.

[0005] In implementing bioremediation, it is judged whether a contaminated site can be treated with biostimulation or should be treated with bioaugmentation in which microorganisms having the capability to degrade contaminants are introduced from outside of a system, and it has been desired that such judgment be performed promptly (JP 2000-079000 A, for example).

Disclosure of Invention

Problem to be Solved by the Invention

[0006] With the foregoing in mind, it is an object of the present invention to provide a method whereby a microorganism in an environment contaminated with tetrachloroethylene (PCE) and trichloroethylene (TCE) is detected and the capability of the environment to degrade contaminants (a biological activity) can be judged promptly, and a polynucleotide for detecting a microorganism used in the judging method.

Means for Solving Problem

[0007] In order to achieve the above-described object, a method of judging a biological activity in an environment according to the present invention is a method of judging a biological activity in an environment contaminated with

an organochlorine compound that is at least one of PCE and TCE. The method includes: amplifying a nucleic acid extracted from an environmental sample by a gene amplification method so as to use the nucleic acid as a target; hybridizing the target to at least one DNA probe including a base sequence unique to each of at least one type of bacterium related to degradation of the organochlorine compound so that the at least one type of bacterium in the environment is detected; and judging the capability of the environment to eliminate the organochlorine compound based on the degrading capability of the at least one type of bacterium that is detected with respect to the organochlorine compound and a dechlorinated product of the organochlorine compound.

The at least one DNA probe includes one or more DNA probes containing a polynucleotide according to the present invention, i.e. any one of the types of polynucleotides described below in (1) to (4).

(1) A polynucleotide comprising any one of base sequences represented by SEQ ID NOS: 1 to 17 and SEQ ID NOS: 19 to 105 of the Sequence Listing, respectively.

(2) A polynucleotide comprising a base sequence obtained by deletion, substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which is hybridizable to a polynucleotide comprising a base sequence complementary to the polynucleotide described in (1) under a stringent condition.

(3) A polynucleotide comprising a base sequence obtained by deletion, substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which has a homology of 90% or higher with the polynucleotide described in (1).

(4) A polynucleotide comprising a base sequence complementary to any one of the polynucleotides described in (1) to (3).

Furthermore, the at least one type of bacterium related to degradation of the organochlorine compound that is to be detected in the

present invention includes one or more types of anaerobic bacteria selected from a group consisting of types of bacteria denoted below as A to R.

A: Dehalospirillum multivorans

B: Desulfitobacterium frappieri

C: Actinomycetales Sm-1 (Rhodococcus sp. Sm−1)

D: Rhodococcus rhodococcus

E: Xanthobacter flavus

F: Mycobacterium L1

G: Desulfomicrobium norvegicum (Desulfovibrio baculatus)

H: Desulfitobacterium dehalogenans

I: Desulfitobacterium hafniense

J: Clostridium formicoaceticum

K: Desulfuromonas chloroethenica

L: Acetobacterium woodii DSM 1030

M: Dehalobacter restrictus

N: Desulfitobacterium sp. strain PCE1

O: Desulfitobacterium frappieri TCE1

P: Acetobacterium woodii DSM 2396

Q: Desulfomonile tiedjei DCB−1

R: Dehalococcoides ethenogenes 195

Effects of the Invention

[0008] In relation to bioremediation with respect to an environment contaminated with an organochlorine compound such as PCE, TCE or the like, if an anaerobic bacterium capable of degrading the organochlorine compound and a dechlorinated product thereof can be detected promptly from the contaminated environment, an assessment of whether biostimulation can be performed or bioaugmentation should be performed is facilitated. The inventors of the present invention took note of this fact and conducted vigorous studies on a method of detecting the currently known 18 types of anaerobic bacteria capable of degrading organochlorine compounds and

dechlorinated products thereof (the types of bacteria denoted as A to R; hereinafter, referred to also as anaerobic bacteria related to degradation of organochlorine compounds).

[0009] As a result of the studies, the inventors found that each of the types of bacteria denoted as A to R has specificity in terms of base sequences in an ITS region (SEQ ID NOS: 1 to 18 of the Sequence Listing) that is a genome region common to the types of bacteria denoted as A to R, and the use of a DNA probe containing a polynucleotide based on the base sequences allows each of the types of bacteria denoted as A to R to be detected using a genetic detection technique such as, for example, a DNA microarray.

[0010] The inventors of the present invention conducted further studies to determine, based on the base sequences in the respective ITS regions of the 18 types of bacteria denoted as A to R (SEQ ID NOS: 1 to 18 of the Sequence Listing), specific base sequences of a length that can be used suitably for, for example, a DNA microarray, with which the types of bacteria denoted as A to R can be detected at the same time without cross-reacting with one another (SEQ ID NOS: 19 to 115 of the Sequence Listing), thus arriving at the present invention.

[0011] The ITS region refers to a transcribed region between a 16S ribosomal DNA and a 23S ribosomal DNA of a bacteria genome occurs (Internal Transcribed Spacer). The base sequences in the respective ITS regions of the types of bacteria denoted as A to Q (assigned SEQ ID NOS: 1 to 17, respectively) were determined for the first time by the inventors of the present invention.

[0012] According to the present invention, a bacterium related to degradation of an organochlorine compound in a contaminated environment is detected promptly using a DNA probe, and based on the degrading capability of the bacterium (see, for example, FIG. 5B), the capability of the environment to eliminate PCE and a dechlorinated product thereof can be judged. Therefore, according to the present invention, in implementing

bioremediation, an assessment of whether biostimulation can be performed or bioaugmentation should be performed is facilitated, thereby allowing the selection of a proper method of remedying an environment. Moreover, since a method of remedying an environment is selected promptly and properly, remediation of the environment can be performed at low cost.

[0013] It is thought that PCE hardly is degraded under an aerobic condition. Further, generally, an environment 50 cm or more below the surface of the Earth is believed to be anaerobic. Therefore, in remedying an environment contaminated with PCE or a contaminated environment 50 cm or more below the surface of the Earth, the use of an aerobic microorganism instead of an anaerobic microorganism that essentially is applicable may incur extra cost and energy consumption. The present invention can avoid incurring such extra cost and energy use.

Brief Description of Drawings

[0014] [FIG. 1] FIG. 1 is a diagram showing results of detection using a DNA microarray performed in Example 1.

[FIG. 2A] FIG. 2A is a photograph showing results of scanning a DNA microarray in Example 2.

[FIG. 2B] FIG. 2B is a graph showing a result of detecting a bacterium M using the DNA microarray in Example 2.

[FIG. 3A] FIG. 3A is a photograph showing results of scanning a DNA microarray in Example 3.

[FIG. 3B] FIG. 3B is a graph showing a result of detecting a bacterium J using the DNA microarray in Example 3.

[FIG. 4] FIG. 4 shows photographs showing results of scanning a DNA microarray in Example 4, in which bacteria A, B, I, J, M, N and O were detected, respectively.

[FIG. 5A] FIG. 5A is a diagram illustrating a degradation route of PCE.

[FIG. 5B] FIG. 5B is a diagram explaining degradation activities of

bacteria A to R with respect to organochlorine compounds.

Description of the Invention

[0015] Bacteria to be detected in the biological activity judging method according to the present invention are bacteria related to degradation of organochlorine compounds and include one or more types of bacteria among the 18 types denoted as A to R that are currently known to be involved in degradation of PCE into ethene or carbon dioxide. Furthermore, the bacteria related to degradation of organochlorine compounds are not limited thereto and include bacteria that will be identified in the future to be involved in degradation of PCE.

[0016] The types of bacteria denoted as A to Q are assigned the following deposit numbers by either of ATCC and DSMZ that are organizations for conservation of living resources and are available from the corresponding one of the organizations.

A:	DSM	12446	B:	DSM	13498	C:	ATCC	51239
D:	ATCC	21197	E:	DSM	10330	F:	DSM	6695
G:	DSM	1741	H:	DSM	9161	I:	DSM	10644
J:	ATCC	27076	K:	DSM	12431	L:	DSM	1030
M:	DSM	9455	N:	DSM	10344	O:	DSM	12704
P:	DSM	2396	Q:	ATCC	49306			

[0017] PCE is dechlorinated to TCE, then to dichloroethylene (DCE), and further to vinyl chloride (VC), and in a final stage, degraded into ethene or carbon dioxide. FIG. 5A shows a typical degradation route of PCE. Degradation activities of the types of bacteria denoted as A to R with respect to PCE and dechlorinated products thereof have already been known, and the respective biological activities of these types of bacteria are shown in FIG. 5B. Thus, if at least one of the types of bacteria denoted as A to R can be detected in an environment, it can be judged that the environment is provided with a biological activity corresponding to a degradation activity of the at least one of the types of bacteria thus detected.

[0018] Concretely, for example, in the case where at least one of the types of bacteria denoted as J, L and P is detected in an environment, it can be judged

that the environment has a biological activity of degrading PCE into TCE. Further, in the case where at least one of the types of bacteria denoted as A, G and M is detected, it can be judged that the environment has a biological activity of degrading PCE into cis-dichloroethylene (cisDCE). Further, in the case where at least one of the types of bacteria denoted as B, I, H, N, O and Q is detected, it can be judged that the environment has a biological activity of degrading PCE and TCE into cisDCE. Further, in the case where the bacterium K is detected, it can be judged that the environment has a biological activity of degrading PCE and TCE into DCE. Further, in the case where the bacterium R is detected, it can be judged that the environment has a biological activity of degrading PCE, TCE, DCE and VC into ethene. Further, in the case where at least one of the types of bacteria denoted as C, D and E is detected, it can be judged that the environment has a biological activity of degrading DCE and VC into carbon dioxide. Further, in the case where the bacterium F is detected, it can be judged that the environment has a biological activity of degrading VC into carbon dioxide. Moreover, in the case where two or more types of bacteria that have different degradation activities are detected, it can be judged that the environment has a biological activity as a combination of the respective degradation activities.

[0019] A method of detecting a bacterium in the biological activity judging method according to the present invention includes steps of: extracting a nucleic acid from an environmental sample and producing a target by a gene amplification method; and hybridizing the target to a DNA probe specific to a bacteria to be detected.

[0020] In the present invention, the DNA probe is derived from an ITS region of a bacterium to be detected. That is, a polynucleotide entirely or partly including one of the base sequences represented by SEQ ID NOS: 1 to 18 of the Sequence Listing can be used as the DNA probe in the present invention.

[0021] Generally, a 16SrRNA, a 23SrRNA and a 5SrRNA that are ribosomal

RNAs (rRNAs) of prokaryotes are transcribed as one transcription unit (operon), and therefore, a 16SrRNA gene and a 23 SrRNA gene are located adjacently on a genome. A region between these genes, i.e. a 16SrRNA gene and a 23SrRNA gene is a so-called 16S–23S Internal Transcribed Spacer (ITS) region.

[0022] The inventors of the present invention determined, with respect to the types of bacteria denoted as A to Q, base sequences in the respective ITS regions (assigned SEQ ID NOS: 1 to 17 of the Sequence Listing, respectively) and found for the first time that a polynucleotide having a base sequence within each of these ITS regions can be used to produce a DNA probe unique to each of the types of bacteria denoted as A to Q. The genome base sequence of the bacterium R is determined by Dr. Zinder of Cornell University. Since the base sequence in an ITS region of the bacterium R (SEQ ID NO: 18 of the Sequence Listing) also is specific to the bacterium R, when a DNA probe derived from the ITS region of the bacterium R is used in combination with DNA probes derived respectively from the ITS regions of the 17 types of bacteria denoted as A to Q, the DNA probes can be used as a group of DNA probes that allows all the 18 types of bacteria denoted as A to R to be detected.

[0023] As a DNA probe for detecting the types of bacteria denoted as A to R, a DNA probe including a part of an ITS sequence is used more preferably than a DNA probe including the entire ITS sequence. This is because, in general, the sequence specificity of a DNA probe increases with decreasing length of the DNA probe, thus providing improved reliability. Meanwhile, it is necessary that a sequence itself is unique to each of the types of bacteria. A DNA probe has a length of, for example, 10 bases to the entire ITS sequence, and preferably 40 to 80 bases, though there is no limitation thereto.

[0024] As a DNA probe for detecting the types of bacteria denoted as A to R, a polynucleotide can be used that is derived from an ITS region and has a length of 40 bases, and a concrete example of such a polynucleotide comprises

base sequences represented by SEQ ID NOS: 19 to 115 of the Sequence Listing.

[0025] That is, a polynucleotide including one of the base sequences represented by SEQ ID NOS: 19 to 25 of the Sequence Listing can be used as a DNA probe for detecting the bacterium A; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 26 to 30 of the Sequence Listing can be used as a DNA probe for detecting the bacterium B; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 31 to 35 of the Sequence Listing can be used as a DNA probe for detecting the bacterium C; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 36 to 40 of the Sequence Listing can be used as a DNA probe for detecting the bacterium D; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 41 to 45 of the Sequence Listing can be used as a DNA probe for detecting the bacterium E; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 46 to 48 of the Sequence Listing can be used as a DNA probe for detecting the bacterium F: a polynucleotide including one of the base sequences represented by SEQ ID NOS: 49 to 53 of the Sequence Listing can be used as a DNA probe for detecting the bacterium G; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 54 to 57 of the Sequence Listing can be used as a DNA probe for detecting the bacterium H; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 58 to 62 of the Sequence Listing can be used as a DNA probe for detecting the bacterium I; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 63 to 68 of the Sequence Listing can be used as a DNA probe for detecting the bacterium J; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 69 to 74 of the Sequence Listing can be used as a DNA probe for detecting the bacterium K; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 75 to 79 of the Sequence Listing can be used as a DNA probe for

detecting the bacterium L; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 80 to 86 of the Sequence Listing can be used as a DNA probe for detecting the bacterium M; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 87 to 91 of the Sequence Listing can be used as a DNA probe for detecting the bacterium N; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 92 to 96 of the Sequence Listing can be used as a DNA probe for detecting the bacterium O; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 97 to 99 of the Sequence Listing can be used as a DNA probe for detecting the bacterium P; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 100 to 105 of the Sequence Listing can be used as a DNA probe for detecting the bacterium Q; a polynucleotide including one of the base sequences represented by SEQ ID NOS: 106 to 115 of the Sequence Listing can be used as a DNA probe for detecting the bacterium R.

[0026] With respect to each of the above-described types of polynucleotides comprising the base sequences SEQ ID NOS: 1 to 115 of the Sequence Listing, respectively, even a polynucleotide comprising a base sequence obtained by deletion, substitution or insertion of one to several bases therein can be used as a DNA probe for the biological activity judging method according to the present invention. That is, the DNA probe according to the present invention is a probe containing any one of types of polynucleotides described below in (1) to (4).

(1) A polynucleotide comprising any one of the base sequences represented by SEQ ID NOS: 1 to 115 of the Sequence Listing, respectively.

(2) A polynucleotide comprising a base sequence obtained by deletion, substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which is hybridizable to a polynucleotide comprising a base sequence complementary to the polynucleotide described in (1) under a stringent condition.

(3) A polynucleotide comprising a base sequence obtained by deletion, substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which has a homology of 90% or higher with the polynucleotide described in (1).

(4) A polynucleotide comprising a base sequence complementary to any one of the polynucleotides described in (1) to (3).

[0027] The number of bases that can be deleted, substituted or inserted is, for example, as follows. That is, with respect to 40 bases, the number of bases that can be deleted/inserted is 1 to 6, preferably 1 to 3, and more preferably 1 to 2, and the number of bases that can be substituted is 1 to 4, preferably 1 to 2, and more preferably 1. Further, hybridization under a stringent condition means that two DNA fragments are hybridized to each other under a standard hybridization condition as described by Sambrook J. et al. (Expression of cloned genes in E. coli (Molecular Cloning: A laboratory manual (1989)) Cold Spring harbor Laboratory Press, New York, USA, 9. 47–9. 62 and 11.45–11.61). More concretely, it means that hybridization and washing (for example, in about 2.0 × SSC at 50°C) are performed with reference to ±10°C of a Tm value. Further, the homology is, for example, 90% or higher, preferably 95% or higher, and more preferably 97.5% or higher.

[0028] A gene amplification method for preparing a target to be hybridized to the DNA probe is not limited particularly as long as an ITS region of a bacterium to be detected can be amplified by the method, and as the method, for example, a PCR method can be employed. Since the DNA probe in the present invention is derived from an ITS region of a bacterium, for example, in one gene amplification reaction using a combination of a small number of primers, ITS regions as targets of all the types of bacteria to be detected can be amplified.

[0029] For example, the ITS regions of the types of bacteria denoted as A to Q can be amplified by the use of a polynucleotide comprising a base sequence

represented by SEQ ID NO: 116 of the Sequence Listing as a sense primer and a polynucleotide comprising a base sequence represented by SEQ ID NO: 117 of the Sequence Listing as an antisense primer. Moreover, in the case of amplifying the ITS region of the bacterium R as well at the same time, a polynucleotide comprising a base sequence represented by SEQ ID NO: 118 of the Sequence Listing as an antisense primer further should be added to a reaction solution.

[0030] A detection method by hybridization of a target amplified in the above-described manner to the DNA probe is not limited particularly, and as the method, for example, a conventionally known gene detection technique such as a Southern blotting method, a DNA array method, a DNA microarray method, or a DNA chip method can be used. Among these, a method in which a DNA probe corresponding to a bacterium to be detected is immobilized allows bacteria in all environmental samples to be detected at one time and thus is preferable. Preferably, the target and the DNA probe are labeled suitably depending on the detection method. For example, the target may be labeled at the same time that gene amplification for preparing the target is performed. There is no particular limitation to the labeling, and, for example, fluorescence labeling or RI labeling can be employed.

[0031] An environment as a subject of biological activity judgment according to the present invention is not limited particularly and can be, for example, soil, groundwater, pond water, or seawater that is contaminated with at least one of PCE and TCE. A nucleic acid to be used as a template of the target can be extracted from the environmental sample by a conventionally known method such as, for example, using a commercially available nucleic acid extracting kit without any particular limitation. As the nucleic acid, for example, a DNA or a RNA may be used.

[0032] A bioremediation method according to the present invention is a method of bioremediation with respect to an environment contaminated with an organochlorine compound that is at least one of PCE and TCE, and

includes steps of: performing the method of judging a biological activity in an environment according to the present invention; and stimulating, when a bacterium related to degradation of the organochlorine compound is detected by the method, growth and/or an activity of the bacterium so as to enhance the degradation of the organochlorine compound or a dechlorinated product thereof. In the bioremediation, the capability of an environment to degrade and eliminate an organochlorine compound such as PCE or the like is grasped beforehand by the biological activity judging method according to the present invention, and thus it becomes possible to select a bioremediation method so that the accuracy and promptness of bioremediation can be increased. For example, if the bacterium R is detected, it is expected that PCE can be degraded into ethene using the bacterium R, and thus based on this, biostimulation in which nutrients for enhancing growth and an activity of the bacterium R is introduced into an environment can be selected. Further, for example, similarly in the case where the bacteria K and C are detected, it is expected that PCE can be degraded into carbon dioxide using the two types of bacteria (see FIG. 5B), and thus based on this, biostimulation in which nutrients for enhancing growth and activities of the two types of bacteria are introduced into an environment can be selected.

[0033] A bioremediation method according to another aspect of the present invention is a method of bioremediation with respect to an environment contaminated with an organochlorine compound that is at least one of PCE and TCE, and includes steps of: performing the method of judging a biological activity in an environment according to the present invention; and adding at least one of types of bacteria related to degradation of the organochlorine compound other than a detected bacterium to the environment so as to enhance the degradation of the organochlorine compound or a dechlorinated product thereof. For example, in the case where only the bacterium K is detected, the addition of the bacterium C to the environment allows PCE to be degraded into carbon dioxide.

[0034] A device for detecting a bacterium according to the present invention is a device that can be used in the biological activity judging method according to the present invention, and includes the DNA probe according to the present invention. The detection device according to the present invention is not limited particularly as long as the device includes the DNA probe that is immobilized and can detect the target hybridized to the DNA probe. In the detection device according to the present invention, preferably, at least two such DNA probes are included, and at least two of the above-described types of bacteria can be detected at the same time. More preferably, the detection device includes a polynucleotide including one of the base sequences represented by SEQ ID NOS: 19 to 115 of the Sequence Listing as a DNA probe and can detect all of the types of bacteria denoted as A to R at the same time.

[0035] A DNA microarray according to the present invention is a DNA microarray that can be used in the biological activity judging method according to the present invention, and includes a substrate on which at least one DNA probe according to the present invention is immobilized. In the DNA microarray according to the present invention, preferably, two or more DNA probes according to the present invention are immobilized, and two or more of the above-described types of bacteria can be detected. In order to suppress noise, preferably, the length of the DNA probe(s) immobilized to the DNA microarray according to the present invention is as short as possible, and in order to suppress cross-hybridization by keeping a Tm value constant, preferably, the DNA probes have the same length. There is no particular limitation to the substrate used in the DNA microarray according to the present invention, and as the substrate, a commercially available substrate for a DNA microarray or the like can be used, and the DNA probe(s) can be immobilized to the substrate by a conventionally known method with no particular limitation.

[0036] A detecting kit according to the present invention is a kit for detecting

a bacterium that can be used in the biological activity judging method according to the present invention, and includes: the DNA probe according to the present invention; and a primer for gene amplification and a reagent for gene amplification that are used for preparing a target to be hybridized to the DNA probe so as to be detected. A kit according to another aspect of the present invention includes the DNA microarray according to the present invention instead of the DNA probe according to the present invention. As the primer for gene amplification, for example, a polynucleotide including the base sequence represented by SEQ ID NO: 116 of the Sequence Listing can be used as a sense primer, and a polynucleotide(s) including the base sequence(s) represented by SEQ ID NO(S): 117 and/or 118 of the Sequence Listing can be used as a antisense primer. As the reagent for gene amplification, a conventionally known reagent such as, for example, a buffer, polymerase, or nucleotide can be used. When necessary, the detection kit according to the present invention may include, for example, a reagent for extracting a nucleic acid and a filter or a chip that are used for preparing a target.

[0037] A polynucleotide according to the present invention is a new type of polynucleotide that can be used as a DNA probe for detecting the types of bacteria denoted as A to Q in the biological activity judging method according to the present invention, and is any one of types of polynucleotides described below in (1) to (4). The number of bases to be deleted, substituted or inserted, a stringent condition and a homology are defined in the same manner as in the foregoing description.

(1) A polynucleotide comprising any one of base sequences represented by SEQ ID NOS: 1 to 17 and SEQ ID NOS: 19 to 105 of the Sequence Listing, respectively.

(2) A polynucleotide comprising a base sequence obtained by deletion, substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which is hybridizable to a polynucleotide comprising a base sequence complementary to the polynucleotide described in

(1) under a stringent condition.

(3) A polynucleotide comprising a base sequence obtained by deletion, substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which has a homology of 90% or higher with the polynucleotide described in (1).

(4) A polynucleotide comprising a base sequence complementary to any one of the polynucleotides described in (1) to (3).

[0038] Hereinafter, the present invention will be described by way of examples.

Examples

Example 1

[0039] (Production of a DNA probe and a DNA microarray that allow 18 types of bacteria to be detected at the same time)

1. Production of DNA probe

A base sequence having 40 bases was designed based on the ITS sequences of the types of bacteria denoted as A to R (assigned SEQ ID NOS: 1 to 18 of the Sequence Listing, respectively) and used as a DNA probe. In designing the DNA probe, a precondition was set that the DNA probe was a single-stranded probe of 40 bases having a GC content of 48 to 50%, exhibited no or almost no complementation, and was not found in the International Nucleotide Sequence Database GenBank (if any, contained two or more mispairs). The following are DNA probes thus produced (of 3 to 10 types for each type of bacterium) and sequence ID numbers of their respective base sequences (SEQ ID NOS: 19 to 115 of the Sequence Listing).

[0040] Probes A1 to A7 for the bacterium A have the base sequences represented by SEQ ID NOS: 19 to 25 of the Sequence Listing, respectively; probes B1 to B5 for the bacterium B have the base sequences represented by SEQ ID NOS: 26 to 30 of the Sequence Listing, respectively; probes C1 to C5 for the bacterium C have the base sequences represented by SEQ ID NOS: 31 to 35 of the Sequence Listing, respectively; probes D1 to D5 for the bacterium

D have the base sequences represented by SEQ ID NOS: 36 to 40 of the Sequence Listing, respectively; probes E1 to E5 for the bacterium E have the base sequences represented by SEQ ID NOS: 41 to 45 of the Sequence Listing, respectively; probes F 1 to F3 for the bacterium F have the base sequences represented by SEQ ID NOS: 46 to 48 of the Sequence Listing, respectively: probes G1 to G5 for the bacterium G have the base sequences represented by SEQ ID NOS: 49 to 53 of the Sequence Listing, respectively; probes H1 to H4 for the bacterium H have the base sequences represented by SEQ ID NOS: 54 to 57 of the Sequence Listing, respectively; probes I1 to I5 for the bacterium I have the base sequences represented by SEQ ID NOS: 58 to 62 of the Sequence Listing, respectively; probes J1 to J6 for the bacterium J have the base sequences represented by SEQ ID NOS: 63 to 68 of the Sequence Listing, respectively; probes K1 to K6 for the bacterium K have the base sequences represented by SEQ ID NOS: 69 to 74 of the Sequence Listing, respectively; probes L1 to L5 for the bacterium L have the base sequences represented by SEQ ID NOS: 75 to 79 of the Sequence Listing, respectively; probes M1 to M7 for the bacterium M have the base sequences represented by SEQ ID NOS: 80 to 86 of the Sequence Listing, respectively; probes N1 to N5 for the bacterium N have the base sequences represented by SEQ ID NOS: 87 to 91 of the Sequence Listing, respectively; probes O1 to O5 for the bacterium O have the base sequences represented by SEQ ID NOS: 92 to 96 of the Sequence Listing, respectively; probes P1 to P3 for the bacterium P have the base sequences represented by SEQ ID NOS: 97 to 99 of the Sequence Listing, respectively; probes Q1 to Q6 for the bacterium Q have the base sequences represented by SEQ ID NOS: 100 to 105 of the Sequence Listing, respectively; probes R1 to R10 for the bacterium R have the base sequences represented by SEQ ID NOS: 106 to 115 of the Sequence Listing, respectively.

[0041]

2. Production of DNA microarray and confirmation of specificity of DNA probe

Next, using an Affymetrix 417 Arrayer (manufactured by Affymetrix, Inc.), each of the above-described 97 types of DNA probes was printed in a customized manner on a TaKaRa-Hubble Slide Glass (manufactured by Takara Bio Inc.) to produce a DNA microarray. Then, a target was prepared using each of the types of bacteria denoted as A to R and hybridized to the DNA microarray so as to be used to confirm the specificity of the DNA probes.

[0042] The target was prepared in a manner that an ITS region of each of the above-described types of bacteria was amplified by a PCR method. In performing the PCR, as a sense primer, a non-labeled primer having the base sequence represented by SEQ ID NO: 116 of the Sequence Listing was used. Further, as an antisense primer, a Cy3-labeled primer having the base sequence represented by SEQ ID NO: 117 of the Sequence Listing was used for the bacteria other than the bacterium R, and a Cy3-labeled primer having the base sequence represented by SEQ ID NO: 118 of the Sequence Listing was used for the bacterium R. Reaction conditions for the PCR were set so as to conform to a standard protocol.

[0043] An amplified product to be used as a target resulting from the PCR was desalted using an AutoSeq G−50 (manufactured by Pharmacia Corporation), then vacuum-dried using a SpeedVac (manufactured by Savant Instrument, Inc.) and then dissolved in a buffer of 5 × SSC, 0.2% SDS and 50% formamide at their respective final concentrations. A target solution thus obtained was boiled at a temperature of 94°C for 3 minutes, then cooled with ice for at least two minutes, and then applied on the DNA microarray. The DNA microarray was covered with a cover glass and then placed in a hybridization chamber at a set temperature of 42°C for at least 4 hours. After that, the DNA microarray was washed with 0.2 × SSC and 0.2% SDS for 5 minutes, with 0.2 × SSC for 5 minutes, and with 0.05 × SSC for several seconds, and then spin-dried at a speed of 1,800 rpm. Scanning was performed using a ScanArray version 5 (manufactured by PerkinElmer Japan Co., Ltd.) to obtain measurement results.

[0044] The results are shown in FIG. 1. FIG. 1 is a graph showing whether the targets derived from the respective ITS sequences of the 18 types of bacteria denoted as A to R plotted on a vertical axis were hybridized to the 97 DNA probes plotted on a horizontal axis. In the figure, black-painted portions show that the DNA probes were hybridized to the targets, respectively exhibiting fluorescent signals of 500 fluorescent units or more. A phylogenetic tree on the vertical axis was created based on the alignment of the ITS sequences. As shown in FIG. 1, it was proved that the targets prepared using the types of bacteria denoted as A to R were hybridized significantly only to the DNA probes for the types of bacteria denoted as A to R, respectively, without being cross-hybridized.

Example 2

[0045] (Judgment 1 of biological activity in contaminated environment)

Using a FastPrep bead-beater and a soil DNA extraction kit (manufactured by Qbiogene, Inc.) and following instruction manuals, a DNA was extracted from 250 mg of a soil sample provided by Matsushita Environmental & Air-conditioning Engineering. The DNA in an amount of about 1 µl was added to 50 µl of a standard Amplitaq Gold PCR mixture (manufactured by Applied Biosystems) containing a non-labeled sense primer 27F (SEQ ID NO: 116 of the Sequence Listing) and a Cy3-labeled antisense primer 132 R (SEQ ID NO: 117 of the Sequence Listing) or 341R (SEQ ID NO: 118 of the Sequence Listing). After performing PCR in accordance with a standard protocol, a PCR amplified product was desalted using an Autoseq G−50 (manufactured by Pharmacia Corporation) and then vacuum-dried using a SpeedVac (manufactured by Savant Instrument, Inc.). The PCR amplified product thus dried was dissolved in a buffer of 5 × SSC, 0.2% SDS and 50% formamide at their respective final concentrations, and a solution thus obtained was boiled at a temperature of 94°C for 3 minutes, then cooled with ice for at least two minutes, and then applied on the DNA microarray produced in Example 1. The DNA microarray was covered with a cover glass

and then placed in a hybridization chamber at a set temperature of 42°C for at least 4 hours. After that, the DNA microarray was washed with 0.2 × SSC and 0.2% SDS for 5 minutes, with 0.2 × SSC for 5 minutes, and with 0.05 × SSC for several seconds, and then spin-dried at a speed of 1,800 rpm. Scanning was performed using a ScanArray version 5 (manufactured by PerkinElmer Japan Co., Ltd.) to obtain measurement results.

[0046] The results of the scanning of the DNA microarrays are shown partially in FIG. 2. FIG. 2A shows scan images, and FIG. 2B is a graph in which the results are shown quantitatively. As shown in FIG. 2, significant hybridization of the bacterium M (Dehalobacter restrictus DSM 945) to the probes was detected. Thus, it could be judged that the soil as the sample had capability to degrade PCE into cisDCE (See FIG. 5B).

Example 3

[0047] (Judgment 2 of biological activity in contaminated environment)

In this example, 300 ml of a groundwater sample provided by Matsushita Environmental & Air-conditioning Engineering was used instead of the soil sample, and a DNA was extracted from debris obtained by centrifugation at a speed of 7,000 rpm with respect to the groundwater sample. Except for this, in the same manner as in Example 2, bacteria in the groundwater sample were detected using the DNA microarray.

[0048] The results of the detection are shown partially in FIG. 3. FIG. 3A shows scan images, and FIG. 3B is a graph in which the results are shown quantitatively. As shown in FIG. 3, significant hybridization of the bacterium J (Clostridium formicoaceticum ATCC 27076) to the probes was detected. Thus, it could be judged that the soil as the sample had the capability to degrade PCE into TCE (See FIG. 5B).

Example 4

[0049] (Judgment 3 of biological activity in contaminated environment)

In this example, an anaerobic enrichment culture sample provided by Dr. T. H. Lee (the Republic of Korea) was used instead of the soil sample.

Except for this, in the same manner as in Example 2, the bacteria in the sample were detected.

[0050] Results of the detection are shown in FIG. 4. As shown in FIG. 4, strong signals were detected in part of the probes for the bacteria A, J, M, N and O, and weak signals were detected also in the probes for the bacteria B and J. Thus, it could be judged that the sample had capability to convert PCE into cisDCE (see FIG. 5B). These judgment results agreed with the analysis data on PCE/cisDCE in the enrichment culture provided by Dr. T. H. Lee.

Industrial Applicability

[0051] As described in the foregoing discussion, the method of judging a biological activity in an environment according to the present invention and the polynucleotide according to the present invention are useful in the method of remedying an environment contaminated with PCE or the like, particularly, in the field of bioremediation.

Sequence Listing Free Text

[0052]

SEQ ID NO: 116 Sense primer 27F for PCR

SEQ ID NO: 117 Antisense primer 132R for PCR

SEQ ID NO: 118 Antisense primer 341R for PCR

CLAIMS

[1] A method of judging a biological activity in an environment contaminated with an organochlorine compound that is at least one of tetrachloroethylene (PCE) and trichloroethylene (TCE), the method comprising:

amplifying a nucleic acid extracted from an environmental sample by a gene amplification method so as to use the nucleic acid as a target;

hybridizing the target to at least one DNA probe including a base sequence unique to each of at least one type of bacterium related to degradation of the organochlorine compound so that the at least one type of bacterium in the environment is detected; and

judging capability of the environment to eliminate the organochlorine compound based on degrading capability of the at least one type of bacterium that is detected with respect to the organochlorine compound and a dechlorinated product thereof,

wherein the at least one DNA probe includes a DNA probe containing any one of types of polynucleotides described below in (1) to (4), and

the at least one type of bacterium related to degradation of the organochlorine compound is at least one type of an anaerobic bacterium selected from a group consisting of types of bacteria denoted below as A to R:

(1) A polynucleotide comprising any one of base sequences represented by SEQ ID NOS: 1 to 17 and SEQ ID NOS: 19 to 105 of the Sequence Listing, respectively.

(2) A polynucleotide comprising a base sequence obtained by deletion, substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which is hybridizable to a polynucleotide comprising a base sequence complementary to the polynucleotide described in (1) under a stringent condition.

(3) A polynucleotide comprising a base sequence obtained by deletion,

substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which has a homology of 90% or higher with the polynucleotide described in (1).

(4) A polynucleotide comprising a base sequence complementary to any one of the polynucleotides described in (1) to (3).

A: Dehalospirillum multivorans

B: Desulfitobacterium frappieri

C: Actinomycetales Sm-1 (Rhodococcus sp. Sm−1)

D: Rhodococcus rhodococcus

E: Xanthobacter flavus

F: Mycobacterium L1

G: Desulfomicrobium norvegicum (Desulfovibrio baculatus)

H: Desulfitobacterium dehalogenans

I: Desulfitobacterium hafniense

J: Clostridium formicoaceticum

K: Desulfuromonas chloroethenica

L: Acetobacterium woodii DSM 1030

M: Dehalobacter restrictus

N: Desulfitobacterium sp. strain PCE1

O: Desulfitobacterium frappieri TCE1

P: Acetobacterium woodii DSM 2396

Q: Desulfomonile tiedjei DCB−1

R: Dehalococcoides ethenogenes 195.

[2] The method according to claim 1,

wherein the gene amplification method with respect to the nucleic acid uses as a sense primer, a primer that contains a polynucleotide comprising a base sequence represented by SEQ ID NO: 116 of the Sequence Listing, and as an antisense primer, a primer that contains a polynucleotide comprising a base sequence represented by SEQ ID NO: 117 of the Sequence Listing and/or a primer that contains a polynucleotide comprising a base

sequence represented by SEQ ID NO: 118 of the Sequence Listing.

[3] The method according to claim 1,

wherein the contaminated environment is selected from a group consisting of soil, groundwater, pond water and seawater.

[4] The method according to claim 1,

wherein a DNA probe for detecting the bacterium A contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 1 and 19 to 25 of the Sequence Listing.

[5] The method according to claim 1,

wherein a DNA probe for detecting the bacterium B contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 2 and 26 to 30 of the Sequence Listing.

[6] The method according to claim 1,

wherein a DNA probe for detecting the bacterium C contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 3 and 31 to 35 of the Sequence Listing.

[7] The method according to claim 1,

wherein a DNA probe for detecting the bacterium D contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 4 and 36 to 40 of the Sequence Listing.

[8] The method according to claim 1,

wherein a DNA probe for detecting the bacterium E contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 5 and 41 to 45 of the Sequence Listing.

[9] The method according to claim 1,

wherein a DNA probe for detecting the bacterium F contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 6 and 46 to 48 of the Sequence Listing.

[10] The method according to claim 1,

wherein a DNA probe for detecting the bacterium G contains any one

of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 7 and 49 to 53 of the Sequence Listing.

[11] The method according to claim 1,

wherein a DNA probe for detecting the bacterium H contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 8 and 54 to 57 of the Sequence Listing.

[12] The method according to claim 1,

wherein a DNA probe for detecting the bacterium I contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 9 and 58 to 62 of the Sequence Listing.

[13] The method according to claim 1,

wherein a DNA probe for detecting the bacterium J contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 10 and 63 to 68 of the Sequence Listing.

[14] The method according to claim 1,

wherein a DNA probe for detecting the bacterium K contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 11 and 69 to 74 of the Sequence Listing.

[15] The method according to claim 1,

wherein a DNA probe for detecting the bacterium L contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 12 and 75 to 79 of the Sequence Listing.

[16] The method according to claim 1,

wherein a DNA probe for detecting the bacterium M contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 13 and 80 to 86 of the Sequence Listing.

[17] The method according to claim 1,

wherein a DNA probe for detecting the bacterium N contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 14 and 87 to 91 of the Sequence Listing.

[18] The method according to claim 1,

wherein a DNA probe for detecting the bacterium O contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 15 and 92 to 96 of the Sequence Listing.

[19] The method according to claim 1,

wherein a DNA probe for detecting the bacterium P contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 16 and 97 to 99 of the Sequence Listing.

[20] The method according to claim 1,

wherein a DNA probe for detecting the bacterium Q contains any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 17 and 100 to 105 of the Sequence Listing.

[21] The method according to claim 1,

wherein in a case where at least one of the types of bacteria denoted as J, L and P is detected, it is judged that the environment has the capability to degrade PCE into TCE.

[22] The method according to claim 1,

wherein in a case where at least one of the types of bacteria denoted as A, G and M is detected, it is judged that the environment has the capability to degrade PCE into cis-dichloroethylene (cisDCE).

[23] The method according to claim 1,

wherein in a case where at least one of the types of bacteria denoted as B, I, H, N, O and Q is detected, it is judged that the environment has the capability to degrade PCE and TCE into cisDCE.

[24] The method according to claim 1,

wherein in a case where the type of bacterium denoted as K is detected, it is judged that the environment has the capability to degrade PCE and TCE into DCE.

[25] The method according to claim 1,

wherein in a case where the type of bacterium denoted as R is

detected, it is judged that the environment has the capability to degrade PCE, TCE, DCE and vinyl chloride (VC) into ethene.

[26] The method according to claim 1,

wherein in a case where at least one of the types of bacteria denoted as C, D and E is detected, it is judged that the environment has the capability to degrade DCE and VC into carbon dioxide.

[27] The method according to claim 1,

wherein in a case where the type of bacterium denoted as F is detected, it is judged that the environment has the capability to degrade VC into carbon dioxide.

[28] A bioremediation method with respect to an environment contaminated with an organochlorine compound that is at least one of PCE and TCE, the method comprising steps of:

judging a biological activity in the environment by the method according to claim 1; and

stimulating, when a bacterium related to degradation of the organochlorine compound is detected, growth and/or an activity of the bacterium so as to enhance the degradation of the organochlorine compound or a dechlorinated product of the organochlorine compound.

[29] A bioremediation method with respect to an environment contaminated with an organochlorine compound that is at least one of PCE and TCE, the method comprising steps of:

judging a biological activity in the environment by the method according to claim 1; and

adding at least one of types of bacteria related to degradation of the organochlorine compound other than a detected bacterium to the environment so as to enhance the degradation of the organochlorine compound or a dechlorinated product of the organochlorine compound.

[30] A device for detecting the at least one type of bacterium used in the method according to claim 1, comprising as a DNA probe, any one of the types

of polynucleotides described in (1) to (4).

[31] The device according to claim 30,

wherein at least two DNA probes as claimed in claim 30 are included, and at least two of the at least one type of bacterium can be detected at the same time.

[32] A DNA microarray that is used in the method according to claim 1, comprising a substrate on which a DNA probe containing any one of the types of polynucleotides described in (1) to (4) is immobilized.

[33] The DNA microarray according to claim 32,

wherein at least two DNA probes as claimed in claim 32 are immobilized, and at least two of the at least one type of bacterium can be detected at the same time.

[34] A kit for detecting the at least one type of bacterium used in the method according to claim 1, comprising:

a DNA probe containing any one of the types of polynucleotides described in (1) to (4); and

a primer for gene amplification and a reagent for gene amplification that are used for preparing a target to be hybridized to the DNA probe so as to be detected.

[35] A kit for detecting the at least one type of bacterium used in the method according to claim 1, comprising:

the DNA microarray according to claim 32; and

a primer for gene amplification and a reagent for gene amplification that are used for preparing a target to be hybridized to the DNA probe so as to be detected.

[36] A primer that is used as a sense primer in a gene amplification method with respect to a nucleic acid in the method according to claim 1, comprising a polynucleotide comprising a base sequence represented by SEQ ID NO: 116 of the Sequence Listing.

[37] A primer that is used as an antisense primer in a gene amplification

method with respect to a nucleic acid in the method according to claim 1, comprising a polynucleotide comprising a base sequence represented by SEQ ID NO: 117 or 118 of the Sequence Listing.

[38] A DNA probe for detecting the bacterium A that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 1 and 19 to 25 of the Sequence Listing.

[39] A DNA probe for detecting the bacterium B that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 2 and 26 to 30 of the Sequence Listing.

[40] A DNA probe for detecting the bacterium C that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 3 and 31 to 35 of the Sequence Listing.

[41] A DNA probe for detecting the bacterium D that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 4 and 36 to 40 of the Sequence Listing.

[42] A DNA probe for detecting the bacterium E that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 5 and 41 to 45 of the Sequence Listing.

[43] A DNA probe for detecting the bacterium F that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 6 and 46 to 48 of the Sequence Listing.

[44] A DNA probe for detecting the bacterium G that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID

NOS: 7 and 49 to 53 of the Sequence Listing.

[45] A DNA probe for detecting the bacterium H that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 8 and 54 to 57 of the Sequence Listing.

[46] A DNA probe for detecting the bacterium I that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 9 and 58 to 62 of the Sequence Listing.

[47] A DNA probe for detecting the bacterium J that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 10 and 63 to 68 of the Sequence Listing.

[48] A DNA probe for detecting the bacterium K that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 11 and 69 to 74 of the Sequence Listing.

[49] A DNA probe for detecting the bacterium L that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 12 and 75 to 79 of the Sequence Listing.

[50] A DNA probe for detecting the bacterium M that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 13 and 80 to 86 of the Sequence Listing.

[51] A DNA probe for detecting the bacterium N that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 14 and 87 to 91 of the Sequence Listing.

[52] A DNA probe for detecting the bacterium O that can be used in the

method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 15 and 92 to 96 of the Sequence Listing.

[53] A DNA probe for detecting the bacterium P that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 16 and 97 to 99 of the Sequence Listing.

[54] A DNA probe for detecting the bacterium Q that can be used in the method according to claim 1, comprising any one of the types of polynucleotides described in (1) to (4) that corresponds to any one of SEQ ID NOS: 17 and 100 to 105 of the Sequence Listing.

[55] A polynucleotide that can be used as a DNA probe for detecting a bacterium related to degradation of an organochlorine compound in the method according to claim 1, the polynucleotide being any one of types of polynucleotides described below in (1) to (4):

(1) A polynucleotide comprising any one of base sequences represented by SEQ ID NOS: 1 to 17 and SEQ ID NOS: 19 to 105 of the Sequence Listing, respectively.

(2) A polynucleotide comprising a base sequence obtained by deletion, substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which is hybridizable to a polynucleotide comprising a base sequence complementary to the polynucleotide described in (1) under a stringent condition.

(3) A polynucleotide comprising a base sequence obtained by deletion, substitution or insertion of one to several bases in the base sequence of the polynucleotide described in (1), which has a homology of 90% or higher with the polynucleotide described in (1).

(4) A polynucleotide comprising a base sequence complementary to any one of the polynucleotides described in (1) to (3).

ABSTRACT

It is an object to provide a method whereby with respect to a microorganism present in an environment contaminated with tetrachloroethylene (PCE) and trichloroethylene (TCE), capability of the microorganism to degrade contaminants can be judged promptly.

In order to achieve the above-described object, a biological activity judging method according to the present invention is a method of judging capability of a contaminated environment to eliminate an organochlorine compound, in which using a DNA probe that allows a bacterium to be detected specifically, which has a degradation activity with respect to the organochlorine compound and/or a dechlorinated product thereof, the bacterium in the environment is detected.

The present invention also provides a new type of polynucleotide that can be used as a DNA probe in the biological activity judging method according to the present invention.

Sequence Listing

<110> ITO, Yoshitaka
TAKAMIZAWA, Kazuhiro
IWAHASHI, Hitoshi

<120> METHOD OF JUDGING BIOLOGICAL ACTIVITY IN BIOREMEDIATION SITE AND POLYNUCLEOTIDE FOR DETECTING MICROORGANISM TO BE USED THEREIN

<130> 10873.1940USWO

<140> New Application
<141> 2006-08-23

<150> PCT/JP2005/003175
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atccctcagt attgagactt cggtctcgat ctatcggatc tcttcagaaa catcagccgg 120

acataggtgg aaacatcatg atctggcatt ggcgggacac cgccgtcttc gtttctcttt 180

cttcgcggac aagcttgacg cccaggttgc ggtcctttgg actgcgttcc ggtttcgggc 240

ctgtagctca ggtggttaga gcgcacccct gataagggtg aggtcggacg ttcgagtcgt 300

cccaggccca ccaccatcag acagttcttg cctgcgcctc atgtccgaag cttcgcgaac 360

tctcgcctgt ggcatcctgt gatggggcca tagctcagtt gggagagcgc gtgctttgca 420

agcatgaggt cgtcggttcg atcccgtctg gctccaccat tcttcttttc ttgaggaaga 480

tgatggcagg gtggtttgcg ctcggctcct ttgagtgaag gctcttgggg tcttgagcgt 540

cttgtccgcg aatatctgtt tcgcatgttc catcatgccg gtctccggcg gaacatgcac 600

ggctgtatga catcgtgaat agggcattga tcgactgtac cgtggcaaca cggtcgggtc 660

gtggggaagg tggcgacacc tttcgatgcg atcattgggt gctgaccgca ccattgtcga 720

caatgcgaag ctggtctttt caaagaagac gtcgaagccg tccggccggg agcaatcctg 780

gtgcgggcct ctgccgaggg gtgggcatcg acgatgagaa cgatcaagtg tcttaagggc 840

attcggtgga tgccttggcg ctaagaggcg aagaaggacg tgatacgctg cgataagctt 900

cggggagccg cgaatgggct ttgatccgga gatttccgaa tggggcaacc ca 952

<210> 6
<211> 579
<212> DNA
<213> Mycobacterium L1

<400> 6
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atggcccttt cacctgtagt gggtgggggt ctggtgcacg acaagcaaac gaccaggatg 180

gggaccttcc ttgtgggggt tgtctggtgc tgccaaacac actgttgggc tttgagacaa 240

caggcccgtg cccgggtttc cgggtggctc cgcggtggtg gggtcggcgt gttgttgcct 300

cactttggtg gtggggtgtg gtgtttgatt tgtggatagt ggttgcgagc atctagcacg 360

caaatgtggc tctcgaggct ttcgggtctg gggggtgtgt ttgtgtgctt ttgatgtgca 420

gtttcttttt tcgaattggt tttttgtgtt gtaagtgttt aagggcgcat ggtggatgcc 480

ttggcactgg gagccgatga aggacgtggg aggctgcgtt atgcctcggg gagctgtcaa 540

ccgagcgtgg atccgaggat gtccgaatgg ggcaaccca 579

<210> 7
<211> 523
<212> DNA
<213> Desulfomicrobium norvegicum

<400> 7
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ctccaactcg ctatttactt gcaaggtttc ttaccttgtc ggtttagaaa tgggcttgta 120

gctcaggtgg ttagagcgca cgcctgataa gcgtgaggtc ggaagttcaa gtcttcccag 180

gcccaccatt tcttagtggg ggtgtagctc agctgggaga gcgcctgcct tgcacgcagg 240

aggtcatcag ttcgatcctg ttcacctcca ccattttcca actcgacaag aatttatgtt 300

gctagtcttt atcgtcagag tgtcttttga cactatggcg cccaagcata gcagcttgtg 360

atcattgaca gacgaatagg tgaagagaag agagttaaga tgttaagggc atacggtgga 420

tgccttggcg tcaggaggcg atgaaggacg tggaaggctg cgataagcct cggggagccg 480

tcaagcaggc tttgatccgg ggatttccga atggggcaac cca 523

<210> 8
<211> 662
<212> DNA
<213> Desulfitobacterium dehalogenans

<400> 8
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catggtttct cgctagagaa atcatatcct aaggtcgatg ctttgaagaa cgtcacggaa 120

gcaatgaagt gaaacgattc aaagtcggag aagtcttaag agacttctta taggaaactt 180

ggcttgtgtg aagcatgagc agaagccata gttgacttat ccacggagtg gaaaaatgcc 240

gaagaggcaa aacggagcaa tccgtaaagt atgggaaatg aagctgttga agttaaaagc 300

taacttgttg tttagttttg agggaccata aagtcttcta tatgggggta tagctcagct 360

gggagagcac ctgccttgca agcagggggt cagcggttcg atcccgctta cctccaccat 420

aatatatctg gtttctctaa tgtttattat gttctttgaa aactgcacag agaagaagaa 480

aactgtaatt aggataacat ctaaaaccta gaagtggcgg caaaaaacgt ttggtcaagc 540

tactaagggc gtacggtgga tgcctaggcg ctaagagtcg aagaaggacg cggcgagcgg 600

cgaaacgcca cggggagcag taagcatgcc ttgatccgtg gatatccgaa tggggcaacc 660

ca 662

<210> 9
<211> 775
<212> DNA
<213> Desulfitobacterium hafniense

<400> 9
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catgttcact ctggaagtga gcatatccta aggtcgatgc tttgaaggac gtcacggaag 120

agatgaagtg aaacggttca aagctggaga agtctataga gacttcgaag tgccgaagag 180

gcaaagcagg ggaaatctgc ataagatgac cctgaagtcg agtcaaacct gttcaagcgc 240

aagcttactt gttgtttagt tttgagagac cataaagtct tctatgggct tatagctcag 300

ctggttagag cgcacgcctg ataagcgtga ggtcggtggt tcgagtccac ctaggcccac 360

cattattcaa agaggataga gacccgaacc tccaaacaat acttcacgcc agaacatacc 420

taacaggggt gagtattgag aggggagcgg ctcccctctc aacgacatgg gggtatagct 480

cagctggggg agcacctgcc ttgcaagcag ggggtcagcg gttcgatccc gcttacctcc 540

accatcatat actggtttct ctaatgttct ttgaaaactg cacagagaag aaaaaactgt 600

aatttaggat aacatctgaa aaacctgaat gtggcggaga cggttggtca agctactaag 660

ggcgtacggt ggatgcctag gcgctaagag tcgaagaagg acgcggcgag cggcgaaacg 720

ccacggggag cagtaagcat gccttgatcc gtggatatcc gaatggggca accca 775

<210> 10
<211> 422
<212> DNA
<213> Clostridium formicoaceticum

<400> 10
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aaggctttta ctatactgtt taattttgag ggacttttgt ttctcaataa gcagacaacc 120

aaaatcttag attttgtgtt agtcgcttag ttaaaaattc tgtaattcac gacaatagtt 180

ttaaaccaac aaaaaatgaa tggaagaatt tttaacatct atagtctttt agattgttct 240

ttgaaaacta aacaatgata tgagaaaaga aaagctgaag taattcacta aaggtcaagt 300

tattaagggc aaagggtgga tgccttggca ctaggagccg aagaaggacg tggtaagctg 360

cgaaaagcca cggggagctg caagcaagta ttgatccgtg gatgtccgaa tggggaaacc 420

ca 422

<210> 11
<211> 699
<212> DNA
<213> Desulfuromonas chloroethenica

<400> 11
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cctccttact cgtaagagta aaggcatcct ggtcaatccc tcggcatggt ccgagcggat 120

gcccgcaaag catcattgtc tgctatttag ttttgagaga ccagaacctc gcaagaggtt 180

ttttgttctt tgagacaaga cgaacgaagg tggaagtggg ctagtagctc agctggctag 240

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cgatcgctta agtagaagac ttttttatgg tcaagctatt aagggcgtac ggtggatgcc 600

ttggcatcgg gaggcgatga aggacgtggt aagctgcgaa aagcttcggt aagccgctaa 660

acaggctttg acccggagat gtccgaatgg ggaaaccca 699

<210> 12
<211> 391
<212> DNA
<213> Acetobacterium woodii

<400> 12
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acaggaagtc atggtactat tttcttttgt atgaccatct ggttatgcaa aaacagttaa 120

agaaggcatc ttaggatgca ttttttaacg ggacaaatac cggagtagtg gtagcaggtc 180

ccaatcgatc attgaaaaca gcatagtgta taaataaaat tataaaatac aatttcttaa 240

cacgaaaacg taaattatta ggatcaagaa gaaaagagca cagggtgaat gccttggcaa 300

tcagagccga cgaaggacgc gacaagctgc gaaaagctac gtgtaggtgc acataaccgt 360

taaagcgtag atatccgaat ggggcaaccc a 391

<210> 13
<211> 608
<212> DNA

<213> Dehalobacter restrictus

<400> 13

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accgattgaa gctagacttc aatctactcc aaggtcggta cttagagtaa agcagtgcaa 120

actggactga ctctcaagta aggtgagttt agcaatttat ttcttgttgt ttagttttga 180

gtgacctgag cacagtaatg tgtaaaagaa acactcaaat aatgtccata catatcagag 240

attctggtaa gtatggaaaa acatccttgt tctttgaaaa ctgcacaacg agaaaagcag 300

aatgcgaaat gcgaaagtaa agacaacgaa atggcgttca aattctaaag cgcaaaaact 360

taacgttttc gcgcgtggca aatttgaact taggagcatc tatgctccgt caggtaagaa 420

ttactaagcg cataggagac attcaaatca tctataacaa gtcgaggaag aaccagaagg 480

tcaagatata aagggcatac ggtggatgcc ttggcgccaa gagccgaaga aggacgcggt 540

taacagcgaa atgccacggg gagtcgtaag caggcataga tccgtggatg tccgaatggg 600

gaaaccca 608

<210> 14
<211> 689
<212> DNA
<213> Desulfitobacterium sp. strain PCE1

<400> 14

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gcaatgaagt gaaacgattc aaagttggag aagtcttaag agacttctga aagccgaaga 180

ggcaaaacgg agcaatccgt aaagtatgag aaatgaagct gttgaagtta aaagctaact 240

tgttgtttag ttttgaggga ccataaagtc ttctatgggc ttatagctca gctggttaga 300

gcgcacgcct gataagcgtg aggtcggtgg ttcgagtcca cctaggccca ccataaaaga 360

ttgatattgt gggggtatag ctcagctggg agagcacctg ccttgcaagc agggggtcag 420

cggttcgacc ccgcttacct ccaccataat atatctggtt tctctaatgt ttattatgtt 480

ctttgaaaac tgcacagaga agaagaaaac tgtaattagg ataacatcta aaacctagaa 540

gtggcggcaa aaaacgtttg gtcaagctac taagggcgta cggtggatgc ctaggcgcta 600

agagtcgaag aaggacgcgg cgagcggcga aacgccacgg ggagcagtaa gcatgccttg 660

atccgtggat atccgaatgg ggcaaccca 689

<210> 15

<211> 468

<212> DNA

<213> Desulfitobacterium frappieri TCE1

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tcataaggac tcacactgtt ttgtttataa atttgattcg ctgaatttcc agaatcaatc 120

acattgaaat cctttggatt tcaattgtta attgtgcact gtgaaatgcg aattgataac 180

gtgggggtgt agctcagttg ggagagcacc tgccttgcaa gcagggggtc aggagttcga 240

ctctcctcat ctccaccaaa gacattcata gtttaaatta attatgaatt gtttaaactg 300

aacattgaaa actacaaata tacaataaac atgaaatagg tcaagttatt aagggcgtag 360

ggcgaatgcc ttggcaccaa gagccgatga aggacgggat aagcaccgat atgcttcggg 420

gagtcgcaaa tagacattga tccggagatt tccgaatggg gcaaccca 468

<210> 16
<211> 511
<212> DNA
<213> Acetobacterium woodii

<400> 16
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acagggagtc atggtactat tttcttttgt atgaccttta ggttatacaa aaggatcgta 120

gtttctggca attttcttta tttttataaa gatgaaaatt gacataaact gcgttagttt 180

ttacaccgct catgcgctaa cgcttaatga gctgccaaat tgaaaatttg ggtaaaaacg 240

tcaaagtggt cattgaaaac agcatagtgt attaaaaaaa catacaattt cagatgttaa 300

caacataaga aaaacgtaag ttaaaggatc gtagttttag gactacaggc gactgacgaa 360

gttctactgt cagttgttaa ggatcaagaa atgaagggca cagggcggat gccttggcac 420

tcagagccga tgaaggacgc gacaagctgc gaaaagctgc gtgaaggtgc acataaccgt 480

tgaagcgcag atatccgaat ggggcaaccc a 511

<210> 17
<211> 471
<212> DNA
<213> Desulfomonile tiedjei DCB-1

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aaccttagta tccgaacgca cacatctgct attcagttct gagaggttga cgataacggc 120

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cgaataggtt ttagatcgag gatactcata tatttaggca atcaagctac taagggccta 360

cggtggatgc cttggcatcg gaagacgatg aaggacgtgg ttagctgcga taagcctcgg 420

ggagttgcta aacacactgt gatccgggga tttccgaatg ggcaacccca a 471

<210> 18
<211> 847
<212> DNA
<213> Dehalococcoides ethenogenes 195

<400> 18
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cattagctca gctggttaga gcgcagtcct gataagactg aggtccttgg ttcgagacca 420

agatggccca ccataaagct aaaacttagc ataatcaaac gaataaaaat acctgctgat 480

taaccggttt ttcgcgagag aaccggtttt tttataaaga agcaggaaga taatgtctat 540

tatttcattt taggtgaata acctgcgctg caaattggta tagtttagta ttcaccgggt 600

tattgggcgg gcaaaaaaat ctttgtgaaa tgaaaatatt tactttaaaa agactgattg 660

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agaacaggct aaattacttg gcctgcttaa agggcgtttt gagcaaaata tacaccgcca 780

cgagggcatt gtttgggcta aggtgcaaga aaagcttaag gcagataccc ttaaattgtg 840

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<210> 19
<211> 40
<212> DNA
<213> Dehalospirillum multivorans

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<210> 20
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<213> Dehalospirillum multivorans

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<210> 21
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<212> DNA
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<210> 22
<211> 40
<212> DNA
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<400> 22
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<210> 23
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<400> 23
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<210> 24
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<213> Dehalospirillum multivorans

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<210> 25
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<210> 26
<211> 40
<212> DNA
<213> Desulfitobacterium frappieri

<400> 26
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<210> 27
<211> 40
<212> DNA

<213> Desulfitobacterium frappieri

<400> 27
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<210> 28
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<213> Desulfitobacterium frappieri

<400> 28
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<210> 29
<211> 40
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<213> Desulfitobacterium frappieri

<400> 29
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<210> 30
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<213> Desulfitobacterium frappieri

<400> 30

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<210> 31
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<212> DNA
<213> Actinomycetales Sm-1

<400> 31
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<210> 32
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<400> 32
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<210> 33
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<400> 33
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<210> 34
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<210> 35
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<400> 35
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<210> 36
<211> 40
<212> DNA
<213> Rhodococcus rhodococcus

<400> 36
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<210> 37
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<213> Rhodococcus rhodococcus

<400> 37
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<210> 38
<211> 40
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<213> Rhodococcus rhodococcus

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<210> 39
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<213> Rhodococcus rhodococcus

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<210> 41
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<213> Xanthobacter flavus

<400> 41
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<210> 42
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<210> 43
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<210> 45
<211> 40
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<400> 45
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<210> 46
<211> 40
<212> DNA
<213> Mycobacterium L1

<400> 46
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<210> 47
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<400> 47
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<210> 48
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<213> Mycobacterium L1

<400> 48
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<210> 49
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<212> DNA
<213> Desulfomicrobium norvegicum

<400> 49
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<210> 50
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<400> 50

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<210> 51
<211> 40
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<210> 54
<211> 40
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<213> Desulfitobacterium dehalogenans

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<210> 55
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<400> 55
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<210> 57
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<213> Desulfitobacterium dehalogenans

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<400> 59
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<210> 60
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<213> Artificial

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<223> Sense primer 27F for PCR

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<211> 16

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<223> Antisense primer 132R for PCR

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<223> Antisense primer 341R for PCR

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